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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

OFFICE
MAY 20 1998
PATENT & TRADEMARK OFFICE
USA

Applicant	:	Zeicher, Marc	Group Art Unit
Serial No.	:	08/807,500	
Filed	:	February 27, 1997	
For	:	NUCLEOTIDE SEQUENCE FOR TREATING CANCER AND INFECTION	
Examiner	:	G. C. Elliot	

DECLARATION UNDER RULE § 1.132

Hon. Commissioner
of Patents and trademarks
Washington, D.C. 20231

Dear Sir:

I, Dr. Marc ZEICHER, inventor of the above-identified patent application, hereby declare and say:

1. I am familiar with the above-identified application and its prosecution history, including the references Stull, et al, Dupont, et al, and Russel, et al cited by the Examiner.

2. The present invention is related to a nucleotide sequence comprising the nucleotide sequence of a virus belonging to the group of autonomous parvoviruses, and at least one effector nucleotide sequence which either encodes a ribozyme able to destroy the messenger RNA coding for a protein indispensable for the survival of the target cell or encodes an effector polypeptide capable of effecting

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the destruction and the normalization of cancer cells or cells infected by virus, bacteria, or intracellular infectious parasites.

3. The effector sequence comprised in the vector according to the invention is described in the specification on page 13, last paragraph and page 14, second paragraph. Specific examples of effector sequences are described in details on page 14, line 7 to page 16, line 32 (effector gene being a ribozyme), on page 15, line 24 to page 16, line 10 (effector genes activating the immune system), on page 43, line 18 to page 45, line 11 (effector gene coding for an enzyme transforming a non toxic prodrug into a cytotoxic drug) and on page 17, line 5 to page 19, line 2 (effector genes encoding a cytotoxic protein).

4. The various examples 1 to 11, present various constructions having incorporated said effector sequences. The example 10, presents results showing the expression of the CAT and B7 genes (see in particular, Table 1 and Table 2 and the Figures). The example 11, presents results obtained with vector comprising the HSV-TK effector gene. These results are the first examples of a TK-transducible vector that is toxic for cancer cells without affecting normal dividing cell.

5. These in-vitro results can be extrapolated for in-vivo applications. As explained throughout the specification, the HSV-TK mediated cytotoxicity obtained with a vector comprising HSV-TK effector gene is not only restricted to the transduced cell but also affect the neighboring non-transduced cell via release of the phosphorylated gancyclovir through gap junctions existing between contiguous cancer cells (bystander effect). Therefore, the majority of cells in a tumor mass could be eradicated if only a fraction of them expresses the effector gene.

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6. Retroviral vectors having incorporated an HSV-TK effector gene have been used in order to transduce brain tumors. In that restricted clinical situation, where proliferating tumor cells are surrounded by non-dividing neural tissues, retroviral vectors are quite tumor specific (see publication of Culver, et al, 1992 cited in the specification).

The vector according to the invention, is advantageously tumor specific. The example 11 shows that the efficiency and selectivity of cell killing mediated by the vector according to the invention are highly improved compared to known in-vitro and in-vivo results obtained by other vectors. Indeed namely there is a destruction of dividing cancer cells (NBK) without any destruction of their normal counterparts, normal dividing fibroblasts. In that experimental setting, other viral vectors like retroviral, adenoviral, adeno-associated viral vectors are killing also the normal dividing fibroblasts (see last paragraph of page 44 and third paragraph of page 45 and Figures 8 and 9 of the application).

7. In view of the information provided by the specification, the person skilled in the art is able to prepare a nucleotide sequence according to the invention which could be used for allowing in-vitro or in-vivo destruction, or normalization of cancer cells or cells comprising an intracellular infectious parasites.

According to the treatment required, the person skilled in the art may select the most efficient effector sequence which could be used in genetic therapy in the treatment of specific disease (cancer, cells infected by intracellular virus, bacteria or infectious parasites).

8. The publication of Stull, et al, presents a review upon the progress and prospect of ribozyme, antisense and gene therapy. Discloses (page 476, left column

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second full paragraph) "*nucleic acid drugs must overcome several formidable obstacles before they can be widely applied as therapeutics. These obstacles require improving the stability of polynucleotide drugs in biological systems, optimizing the affinity and the efficacy of the drug without reducing its selectivity, and targeting and delivering nucleic acids across cell membranes*".

9. The approach of the present invention takes into account these remarks : in the present viral vectors, the nucleic acid construct is protected from the ubiquitous nucleases present in the serum and in the cell cytoplasm by the viral proteic capsid. The autonomous parvoviral vector enters the cell cytoplasm via an ubiquitous receptor, enters the nucleus and uncoat its proteic capsid in the nucleus where conversion from single strand DNA to double strand DNA occurs. DNA replication and transcription will take place with efficacy only in cancer cells and not in dividing normal cells. The DNA encoding the ribozyme was inserted in the Ase I restriction site in the small intron of the MVMp genome. During the splicing of the mRNAs of MVMp, this ribozyme is released by the spliceosome into a nuclear compartment rich in target mRNA. In order to protect the ribozyme from digestion by the nuclear 3' exonucleases, the Inventor has added in 3' to the ribozyme sequence the transcription terminator sequence of the T7 bacteriophage.

The parvoviral vector containing a 100 bp ribozyme sequence inserted in the small intron of the entire MVMp genomic could still replicate and multiply in malignant cells and reinfect other malignant cells after lysis of the infected cells. In leukemia, the vector could be a very valuable addition to existing therapies. Indeed, leukemic cells are very easily reached via intravenous injection. In human chronic myelocytic leukemia (CML) for instance, the parvoviral vector replicates and is

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transduced at a very high level (1000 times over background) whereas no replication and transduction could be detected in normal hematopoietic cells.

Therefore, the parvoviral vector containing a ribozyme destroying specifically the bcr/abl mRNA could easily reach the CML cells after intravelnous injection, multiply and destroy them and reinfect neighbouring leukemic cells. This processus go on as long as there will be leukemic cells. It should of course be slowed down by an antiviral immune response but CML patients are very often immunodepressed.

It is true that diseases like cancer or intracellular infections like AIDS or malaria, are major health problems which are until now badly treated or cured. However, it is also known that multiple factors may allow the development or regression of said diseases.

10. The aim of the present invention is to provide a new nucleotide sequence which will improve the treatment said diseases by destruction or normalization of cancer cell or cell infected by virus, bacteria or intracellular infectious parasites.

11. Various examples in the specification show that expression of the effector sequence included in the vector according to the invention allows the destruction of the host cells and improve the destruction of the surrounding non-transduced host cells while reducing said killing effect upon normal cells.

Of course, although in every normal/cancer cell combination screened (with the exception of B lymphoma), the Inventor has found a positive ration of expression in cancer cells/expression in normal cells, the ratio varies from 10

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(T lymphoma) to 1000 (fibrosarcoma, chronic myelocytic leukemia) and 10000 (monocytic leukemia).

That variability is not indicative of the unpredictable nature of the art but is the reflect of the huge variability among biological properties of human tumors. The variability is also apparent when therapeutics indices of chemotherapeutic drugs are screened. Although these indices never exceed 2 in the most favourable cases, some tumors are completely resistant. That variability in therapeutic indices did not prevent chemotherapeutic drugs to be used in the most appropriate cases. In the present strategy, where in most cases the therapeutic indices are over 100, it would not be appropriate to reject the vectors on the basis of that variability.

The Inventor acknowledge that the experiments summarized in Table I did not show a very high level of transduction of the B7 immunostimulatory molecule (17% in NBK, 7,6M in MRC5-V1, 2,1% in KMST6 and less than 0.1% in the normal counterparts). However, these experiments were performed with a very low multiplicity of infection (MOI < 0.2). Fig. 5 (A and C) shows the results of experiments performed at a higher MOI with the same vector, the FACS profile indicating that 60% of the NBK cells are expressing the B7 gene.

However, the yield of production could be improved and the person skilled in the art is able to produce cellular contaminant-free stocks of recombinant virus particles with titres up to 5×10^8 Infectious Units (IU) / ml. These amounts were obtained by the Inventor according to the method described in the present patent application and other method already described in the literature and well known by the person skilled in the art.

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12. Various examples in the specification show also that the vector according to the invention may create a "bystander effect" (the majority of cells in a tumor mass can be eradicated if only a fraction of them express the effector gene). In addition, the effector sequence according to the invention can be modified so as to attenuate the cytotoxicity of the protein coded by said effector sequence. Finally, the vector according to the invention may also comprise one or more regulatory sequence transactivable by transactivation factors specific for the condition to be treated or for the affected cell line tissue. Said regulatory sequence are capable of cisactivating the effector sequence (see specification from page 20 to 23) and will be expressed preferably in a specific affected cell tissue without inducing a killing effect upon normal cell of normal tissue.

13. The example present such as a specific expression of an effector cell in a specific cell. As shown in Figure 3, the normal fibroblasts only produce quantity of CAT protein at a detection limit whereas the transformed cells produce abundant quantity thereof.

14. Figure 4 shows that no normal cell exceeds 10 pg/10 microgram of cellular extract protein obtained by the expression of the effector gene (CAT gene). However, except from B-lymphomas, all the transformed cells express substantial quantity of CAT protein.

15. Consequently, the above-identified mechanism will allow an improvement in the treatment of the above-mentioned diseases.

16. The inventor of the present invention is a co-author of Dupont (who was paid by the Inventor's grant and was working under the Inventor's supervision), et al publication. This reference which describes the therapeutic approach according to

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the invention implies designing recombinant parvovirus based vector which can be efficiently packaged into infectious viral particles, retain the parvoviral tropism for tumor cells, and selectively express a potent therapeutic gene such as the one coding for a toxin, a prodrug, or a cytokine in order to increase the intrinsic parvoviral antineoplastic activity.

17. The example 12 of the present specification describes how to obtain a recombinant parvovirus vector that can be efficiently packaged into infectious viral particles. Said vector retains the parvoviral tropism for tumor cell and selectively express a potent therapeutic agent as described in the examples 10 and 11.

18. The inventor is of the opinion that the present specification provides enough guidance and direction for a person skilled in the art in order to obtain an efficient vector which allows the the destruction or normalization of cancer cells or cells infected by virus, bacteria or intracellular infectious parasites.

19. The claims 5 and 16 are preferred embodiment of the present invention which describe nucleotide sequence having incorporated regulatory sequence. The specification of the present application describes constructions having incorporated one or more specific promoters. The introduction of said specific promoter are used to improve the therapeutical property of the vector according to the invention. However, without undue experimentation, the person skilled in the art is able to incorporate in the vector according to the invention other specific nucleotide sequences which will improve the therapeutic property of said vector.

Statement Re Perjury

20. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true,

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and further that these statements were made with the kn wledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated:

By: ZEICHER, Marc

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